

IAI00468-15 Supplemental Material

Figure S1. A Schematic representation of the flagellar regulon, SPI-1 and the regulatory cross talk between them. The transcriptional organization of SPI-1 and the motility-chemotaxis regulon in *Salmonella* is shown. Inactivated genes in *S. Paratyphi A* are shown in grey and regulatory proteins are presented in square boxes. In *S. Paratyphi A*, the absence of FliC resultes in induction of flagella and SPI-1 genes in a FliZ-dependent manner (illustrated in red).

Figure S2. Over secretion of SPI-1 effectors in *fliC* *S. Paratyphi A* is a controlled T3SS-dependent process. *S. Paratyphi A* wild-type (SPA) and its derivatives *fliC*, *invA* and *invAssaRfliC* mutant strains were grown in LB for 5.5 h. All cultures were normalized to the same optical density and their supernatant proteins were precipitated by TCA. 25 µl from each precipitated protein fraction were separated on SDS-12% polyacrylamide gel and stained with Coomassie G-250.

Figure S3. The contribution of FliC, T3SS-1 and FliZ to *S. Paratyphi A* invasion into epithelial cells. *S. Paratyphi A* 45157 and its isogenic strains were grown in LB under microaerophilic conditions and used to infect HeLa cells. Invasion was determined at 2 h p.i using the gentamycin protection assay. Under these conditions, the invasion proportion of the wild-type *S. Paratyphi A* is 2.7% from the infecting inoculum. Data represent the mean and SEM of three biological replicates. Mutant strains are shown relative to the wild-type background. ANOVA with Dunnett's Multiple Comparison Test was used to determine differences between the wild-type and each one of the mutants. ***, p<0.0001.

Table S1. Bacterial strains and plasmids used in the study.

Strain or plasmid	Genotype and description	Reference or source
S. Typhimurium SL1344	wild type Sm ^r <i>xyl hisG rpsL</i>	SGSC
S. Typhimurium <i>fliC</i>	SL1344 Δ <i>fliC</i>	This study
S. Typhimurium <i>flihA</i>	SL1344 Δ <i>flihA</i>	This study
S. Typhimurium <i>flijB</i>	SL1344 Δ <i>flijB</i>	This study
S. Typhimurium <i>fliCflijB</i>	SL1344 Δ <i>fliCflijB</i>	This study
S. Typhimurium LT2		SGSC
S. Typhimurium 14028s		SGSC
S. Typhimurium 73727	Clinical isolate 2002	Human stool
S. Typhimurium 74701	Clinical isolate 2002	Human stool
S. Typhimurium 80609	Clinical isolate 2002	Human stool
S. Typhimurium 82788	Clinical isolate 2003	Human stool
S. Typhimurium 88359	Clinical isolate 2003	Human stool
S. Typhimurium 88894	Clinical isolate 2004	Human stool
S. Typhimurium 92273	Clinical isolate 2004	Human stool
S. Typhimurium 92576	Clinical isolate 2004	Human stool
S. Typhimurium 98666	Clinical isolate 2006	Human blood
S. Typhimurium 103259	Clinical isolate 2006	Human blood
S. Typhimurium 116449	Clinical isolate 2008	Human blood
S. Typhimurium 125904	Clinical isolate 2009	Human stool
S. Paratyphi A 45147	Outbreak strain	(1)
S. Paratyphi A <i>invA</i>	45157 Δ <i>invA</i>	(2)
S. Paratyphi A <i>invG</i>	45157 Δ <i>invG</i>	(2)
S. Paratyphi A <i>fliC</i>	45157 Δ <i>fliC</i>	This study
S. Paratyphi A <i>flihA</i>	45157 Δ <i>flihA</i>	This study
S. Paratyphi A <i>fliZ</i>	45157 Δ <i>fliZ</i>	This study
S. Paratyphi A <i>fliCfliZ</i>	45157 Δ <i>fliCfliZ</i>	This study
S. Paratyphi A <i>fliCinvAssaR</i>	45157 Δ <i>fliCinvAssaR</i>	This study
S. Paratyphi A 9150	Reference sequenced strain	SGSC
S. Paratyphi A AKU 12601	Reference sequenced strain	SGSC

S. Paratyphi A 36056/7	Clinical isolate 2007	Traveler from Nepal
S. Paratyphi A 45842/7	Clinical isolate 2007	Traveler from Nepal
S. Paratyphi A 83698	Clinical isolate 2003	Traveler from India
S. Paratyphi A 83753	Clinical isolate 2003	Traveler from India
S. Paratyphi A 93223	Clinical isolate 2004	Traveler from Romania
S. Paratyphi A 105493	Clinical isolate 2006	Traveler from Thailand & Nepal
S. Paratyphi A 108003	Clinical isolate 2007	Traveler from India
S. Paratyphi A 108599	Clinical isolate 2007	Traveler from India
S. Paratyphi A 113498	Clinical isolate 2008	Traveler from Sri Lanka
S. Paratyphi A 119989	Clinical isolate 2008	Traveler from Thailand & India
S. Paratyphi A 124597	Clinical isolate 2009	Traveler from India
S. Paratyphi A 51190	Clinical isolate 2009	Traveler from Nepal
S. Sendai 55-2461		Center for Disease Control, USA
S. Sendai 82-0291		New Mexico human gall bladder
S. Sendai 74-1035		California human blood
S. Sendai Stk.71		(3)
S. Sendai 83-0431		California human gall bladder
S. Typhi CT18	Reference sequenced strain	SGSC
S. Typhi L2	Clinical isolate (year unknown)	NSRC
S. Typhi 152201	Clinical isolate 2013	NSRC
S. Typhi 152496	Clinical isolate 2013	NSRC
S. Typhi 152599	Clinical isolate 2013	NSRC
S. Typhi 152779	Clinical isolate 2013	NSRC
S. Typhi 153029	Clinical isolate 2013	NSRC
S. Typhi 120130191	Clinical isolate 2012	SMC traveler from India and Thailand
S. Gallinarum (287/91)		SGSC
S. Pullorum		Lab colection
Plasmids		
pKD4		(4)
pKD46		(4)
pCP20		(4)

pWSK29	(5)	
pACYC184		
pDE-fliC _{SPA}	Paratyphi A 45157 <i>fliC</i> cloned into pWSK29	This study
pDE-flhA _{SPA}	S. Paratyphi A 45157 <i>flhA</i> cloned into pWSK29	This study
pDE-fliC _{STM}	S. Typhimurium SL1344 <i>fliC</i> cloned into pWSK29	This study
pDE-flhA _{STM}	S. Typhimurium SL1344 <i>flhA</i> cloned into pWSK29	This study
<i>pDE-fljB</i> _{STM}	S. Typhimurium SL1344 <i>fljB</i> cloned into pWSK29	This study
pDE-fliC2HA _{SPA}	S. Paratyphi A 45157 <i>fliC</i> fused to 2HA tag cloned into pWSK29	This study
pDE-sopB2HA _{SPA}	S. Paratyphi A 45157 <i>sopB</i> fused to 2HA tag cloned into pWSK29	This study
pDE-prgJ2HA _{SPA}	S. Paratyphi A 45157 <i>prgJ</i> fused to 2HA tag cloned into pACYC184	This study
pDE-fliC2HA _{STM}	S. Typhimurium SL1344 <i>fliC</i> fused to 2HA tag cloned into pWSK29	This study
pDE-sopB2HA _{STM}	S. Typhimurium SL1344 <i>sopB</i> fused to 2HA tag cloned into pWSK29	This study
pDE-prgJ2HA _{STM}	S. Typhimurium <i>prgJ</i> fused to 2HA tag cloned into pACYC184	This study

SGSC – *Salmonella* genetic Stock Center the University of Calgary.

NSRC – National Salmonella Reference Center, Jerusalem Israel.

SMC – Sheba Medical Center (bacteriology laboratory).

Table S2. Primers used in the study.

Primer	Sequence 5'-3'
sopB 2HA SacI F	TTTGAGCTCTGCTGGCATACACACACCTG

sopB 2HA XbaI R	TTTTTCTAGAAGATGTGATTAATGAAGAAATG
prgJ 2HA Sal1 F	TTTTGTCGACTTACAAAGGTTACATTTCC
prgJ 2HA BglII R	TTTTAGATCTTGAGCGTAATAGCG
fliC 2HA Sal1 F	TTTTGAGCTCGGCCAGCGGTAGTACTGAC
fliC 2HA BglII R	TTTTTCTAGAACGCAGTAAAGAGAGGACG
fliC STM/SPA K.O F	CCCAATAACATCAAGTTGTAATTGATAAGGAAA GATCATGGCACAGTCTGTAGGCTGGAGCTGCT TC
fliC STM/SPA K.O R	TTGTGTACCACGTGTCGGTGAATCAATGCCGG ATTAACGCAGTAAAGAGGTCCATATGAATATCCT CC
flhA STM/SPA K.O F	TATGAACGAGAAGAATACTGATGGCTAATCTGGT CGCGATGCTGCGCTGTGTAGGCTGGAGCTGCT TC
flhA STM/SPA K.O R	GTCATGCGGATATGACGGTTATCGGAAAGCTCAA GGTCGACAACACCAACGTCCATATGAATATCCTC C
fliZ SPA K.O F	CCGAAAAGTGCCGCACAACGTATAGACTACCAG GAGTTCTCATGACGGTGTGTAGGCTGGAGCTGC TTC
fliZ SPA K.O R	CGTTTCACCAACACGACTCTGCTACATCTTATGC TTTTTTAATATATATCGTCCATATGAATATCCTCC
fljB STM K.O F	TTATGGCACAAAGTAATCAACACTAACAGTCTGTC GCTGCTGACCCCTGTAGGCTGGAGCTGCTTC
fljB STM K.O R	GGCTGAATAAAACGAAATAAATTAAACGTAAACAGA GACAGCACGTTCTGCGGGTCCATATGAATATCCT CC
ssaR SPA K.O F	AAAGCTCTGTACCAATTGCGCCAGTGTCAAGATCC CAACCGCCTGCCTGTAGGCTGGAGCTGCTTC
ssaR SPA K.O F	TGTCTTACCCGATTGCGCTTGCAACTGATTGG TATATTGTTCTGCTTCGTCCATATGAATATCCT CC
rpoD F	GGTCTGACCATCGAACAGGGT
rpoD R	ATCAGACCGATGTTGCCTTC
hilD F	GAGATACCGACGCAACGAC
hilD R	CTGCGCTTCTCTGTGGG
hilA F	ATATGCCGTTCTGGTCATCC
hilA R	GCCCTGTCCGTACAGTGTTC
invF F	TGTCGCACCAAGTATCAGGAG
invF R	ACTCGCAGCGTTACGATC
invA F	TCCACGAATATGCTCCACAAG
invA R	CAGACATGCCACGGTACAAC
sipB F	GTGGGCAAAATACGGAAG
sipB R	CCCGATACATCCCATAATGC
sopB F	GAAAATGGCGAAAAGATATC
sopB R	TCATGATAGGGGGAAAGCAC
sopE2 F	CCGACTACCCATTTCATCG
sopE2 R	GCTTCGCATGTCTGACGAGC
flhD F	TGTTCCGCCTCGGTATCAAC
flhD R	CGCGAATCCTGAGTCAAACG
fliA F	GATTGAATCGCTGCCGGAAC
fliA R	ACTATGCAACTGGCTGACCC
fliZ F	AAACATTCCCACGATCTGC
fliZ R	CGGTAAAGGGGGATTCTG

flgM F	CCTTGAAACCCGTTAGCAC
flgM R	GCCGTTTTAATGCTTCGAC
cheA F	AATGGAAAACCTGCTGGATG
cheA R	CTTAGTGCCCGGGTTCTAC
fliC F	AAGAGAGGACGTTTGC GG
fliC R	AGAATCCGCCTTGTGG

References

1. **Gal-Mor O, Suez J, Elhadad D, Porwollik S, Leshem E, Valinsky L, McClelland M, Schwartz E, Rahav G.** 2012. Molecular and cellular characterization of a *Salmonella enterica* serovar Paratyphi a outbreak strain and the human immune response to infection. *Clin Vaccine Immunol* **19**:146-156.
2. **Elhadad D, McClelland M, Rahav G, Gal-Mor O.** 2014. Feverlike Temperature is a Virulence Regulatory Cue Controlling the Motility and Host Cell Entry of Typhoidal Salmonella. *J Infect Dis* doi:10.1093/infdis/jiu663.
3. **Reeves MW, Evins GM, Heiba AA, Plikaytis BD, Farmer JJ, 3rd.** 1989. Clonal nature of *Salmonella typhi* and its genetic relatedness to other salmonellae as shown by multilocus enzyme electrophoresis, and proposal of *Salmonella bongori* comb. nov. *J Clin Microbiol* **27**:313-320.
4. **Datsenko KA, Wanner BL.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* **97**:6640-6645.
5. **Wang RF, Kushner SR.** 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene* **100**:195-199.